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Recent entomogical enquiry on mosquito fauna in Circeo National Park

Claudio De Liberato[1], Adele Magliano[1], Flavia Farina[1] and Luciano Toma[2]

[1]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy*
[2]*Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate, Istituto Superiore di Sanità, Rome, Italy*

Abstract

The present study was carried out in Circeo National Park (Lazio region, Central Italy), in order to collect data about mosquito (Diptera, Culicidae) fauna in a protected area for biodiversity. From 2003 to 2004 seasonal surveys allowed to collect and to identify 380 larvae and 713 adult mosquitoes in 6 sites. A total of 15 mosquito species belonging to 6 genera were recorded; the most abundant species were *Culex pipens* Linnaeus, 1758 known as the main West Nile virus vector, *Ochlerotatus detritus* (Haliday, 1933) and *Culiseta annulata* (Dhrank, 1776). Present data show a noteworthy number of other mosquito species, even if less abundant, reflecting the considerable environmental richness. Respect to the past collections of Anophelinae mosquitoes carried out in the same area once affected by malaria, the present research represents the first monitoring of the whole Culicidae Family in Circeo National Park, up to now. This paper reports the collected data as a first base for a future checklist in this protected area.

Key words

- Culicidae
- mosquito seasonality
- Circeo National Park
- Lazio
- Italy

INTRODUCTION

Most of the previous scientific studies about Culicidae of Pontina Plain, belonging to the Circeo National Park (about 100 km south of Rome, in Latina province), were focused on anophelic fauna, due to the impact of malaria on human health in the area during the first decades of last century [1-8]. During the past decades in this area, changes in species composition of mosquito fauna were strictly related to reclamations works realized from 1930 to 1940. This situation caused a general decrement in mosquito density and number of species [9-11], due to: a) massive employment of organochlorine insecticides for mosquito control, used since 1946 until 1953; b) hydrological transformations; c) water pollution depending on industrialization and anthropization [8]. In spite of past environmental changes caused by human activity, at present Circeo National Park is recognized as biodiversity conservation area by Ramsar convention, thanks to its environmental richness, with very different habitats in a relatively small territory characterized by dunes, costal lakes, plain forest and Mediterranean maquis [12].

In order to investigate the culicid fauna, in autumn 2002 an inquiry started in natural areas of Circeo National Park and in some farms in the surrounding agricultural areas, in order to collect data about the occurrence of species of health relevance. Results of a two-year entomological surveillance have been now revised and are here reported, in order to improve the knowledge about culicid fauna in the first preliminary checklist of mosquito species in this protected area.

MATERIAL AND METHODS

Study area

Study area is located in Latina province, Lazio region, about 100 km south of Rome, partly within Circeo National Park (covering 8484 hectares) and partly in the surrounding agricultural boundaries. About land use and natural features of the Park, the 56% is covered by wooded areas and half-natural habitats, the 18% by agricultural territories, the 2% by wet lands, the 13% by bodies of surface water and the 11% by artificial territories [12]. Thermo-pluviometric data delineate a mild climate environment of attenuated Thermo-Mediterranean biotopes, with accentuated rainfall in October-December; mean temperature of this area is from 9.5 °C to 17.1 °C, and frosts are uncommon. Relative humidity is high all over the year, wind is frequent (South-Western ones dominating) [13].

Field collection sites

Six collection sites (hereafter indicated by letters from A to F), were selected on the base of representative habitat requirements of the commonest mosquito species [15, 16]. Larval collections were performed in both natural and artificial breeding sites; adult collections were carried out in farms with presence of domestic animals. In sites A-D both adult and larvae collec-

Address for correspondence: Luciano Toma, Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: luciano.toma@iss.it.

tions were carried out; in sites E and F (two farms 2 kilometres far from each other) only adult collections were carried out. A brief description of the field collection sites is reported below.

Site A: "Selva del Circeo" (41°20.68'N, 13°02.29'E). "Selva del Circeo", North-East of Sabaudia town, is the relic of "Selva di Terracina", the forest covering a wide marshland of Pontina Plain before the Integral Reclamation of the area. The peculiarity of this environment led to its inclusion in 1977 in the net of the Man and Biosphere (MAB) UNESCO Programme for the Biosphere Reserves. "Selva del Circeo" is the largest Italian remaining fragment of plain forest, with mesohygrophilous vegetation, rich in canals and ditches and situated on a series of Upper Palaeolithic continental dunes [14]. Clay in the basal profile of soil forms a waterproof layer that prevents water vertical dispersion and produces small temporary basins called "piscine", 50-60 cm deep, in rainy periods forming flooded forest areas.

Site B: "Pantani dell'Inferno" (41°20.17'N, 12°59.41'E). The four coastal lakes (Fogliano, Monaci, Caprolace and Paola), situated back to the litoral dune, are the resting fragments of a Quaternary lagoon situated in front of Pontina Plain. Among the coastal lakes and behind the dune-belt there are wet and marshy areas declared "Wetland of International Interest" by Ramsar Convention. Pantani dell'Inferno, at the South-Eastern end of Caprolace Lake, is a small swampy zone, flooded most of the year. Soil is mostly sandy, with a low rate of silt and clay [14]. Constant supply of residues originating from swamp and aquatic plants causes a high quantity of organic substance (organic black subacid soils). The marsh is also rich in *Gambusia affinis* (Baird and Girard 1853) (Cyprinodontiformes, Poeciliidae), a larvivorous fish efficiently plundering mosquito larvae.

Site C: breeding site "Villa Fogliano", (41°23.98'N, 12°55.02'E). Four old bathtubs used as watering places for buffaloes and collocated in a grazing land nearby Fogliano Lake, constitute the only available breeding site of this place, as well as the only artificial and permanent ones of the whole study.

Site D: "Lago di Fogliano"(41°23.88'N, 12°54.76'E). In this place meteoric waters create temporary puddles flooded most of the year: the breeding site are between a path bordering Fogliano Lake's eastern edge and some buffalo pastures. The road is bordered by eucalyptus tree and small puddles are rich in marsh vegetation and organic matter because of the proximity to buffalo fences.

Site E: farm in "Pontinia" locality (41°25.06'N, 13°02.19'E). This farm is a horse, cattle, pig and chicken breeding place.

Site F: farm in "Bella Farnia" locality (41°22.88'N, 12°59.18'E) is a horse, chicken and goose breeding farm. These farms (E, F), were selected because of the position in the agricultural plain, close to natural and semi-natural areas, between coastline and hills. Both of these farms were bordered by canals and rich in water-containers, suitable for mosquito larval development.

Mosquitoes collection

The study was carried out from October 2003 to December 2004, according to the consultancy of the Medical Entomology Unit of the Istituto Superiore di Sanità (ISS) for entomological collections of adult mosquitoes and larvae. Until the fall 2003 sampling activity was aimed only to record data about the species composition in each site, whereas from November 2003 to March 2004 mosquitoes adults and larvae were sampled monthly and every fortnight from the first week of April to the end of October 2004, in order to investigate the species composition and their seasonal dynamics during the year. In sites A and B one CDC trap was placed, and in site A – D larval catches were carried out by using a standard 350 ml dipper. Two different methods were applied for adult mosquitoes collection in farms (site E and site F): four CDC light traps were installed either outside animal shelters and poultry pens, or inside, near the entrance. Traps were kept operating from dusk to dawn, allowing nocturnal mosquitoes catches. Other catches were performed in the morning, using battery-aspirator to collect mosquitoes resting in poultry pens and animal shelters. The same method was used for occasional outdoor captures on human, close to breeding sites chosen for larval sampling. Adult mosquitoes were transferred to laboratory in ice-cooler, kept refrigerated at -20°C and dry preserved. Each breeding site was visited and sampled by 10-20 dips depending on their size. Larvae collections were returned to laboratory in ice-coolers to prevent them to pupate, and preserved in 70% ethanol.

Mosquitoes identification

Mosquito larvae were identified at Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", while adult specimens were determined at Medical Entomology Unit of Istituto Superiore di Sanità. Mosquitoes were identified by morphology, mounting larvae were mounted on slide and adults were properly set for microscopic observation according to the keys for Italian mosquitoes [15, 16].

RESULTS

During 189 surveys a total of 380 larvae and 713 adults were collected. In total, 15 mosquito species were recorded: *Anopheles maculipennis* Meigen, 1818 s.l., *Anopheles plumbeus* Stephens, 1828, *Aedes vexans* (Meigen, 1830), *Aedes albopictus* (Skuse, 1897), *Ochlerotatus geniculatus* (Oliver, 1791), *Ochlerotatus caspius* (Pallas, 1771), *Ochlerotatus communis* (De Geer, 1776), *Ochlerotatus detritus* (Haliday, 1833), *Ochlerotatus rusticus* (Rossi, 1790), *Culex pipiens* Linnaeus, 1758, *Culiseta longiareolata* (Macquart, 1838), *Culiseta annulata* (Shrank, 1776), *Culiseta litorea* (Schute, 1928), *Culiseta subochrea* (Edwards, 1921), *Uranotaenia unguiculata* (Edwards, 1713). Among such species, 4 were recorded as adults only: *An. maculipennis* (in sites E), *Oc. caspius* (in site A, F), *Oc. geniculatus* (in site A), *An. plumbeus* (in site A); 5 species were recorded as larvae only: *Ur. unguiculata* (in sites B, C), *Cs. longiareolata* (in site C), *Cs. litorea* (in site A), *Cs. subochrea* (in site A, C), *Oc. communis* (in site D) (*Table 1*). Seasonal trends of the three most abundant species, *Oc. detritus, Cx. pipens* and *Cs. annulata,* are shown in *Figure 1 and 2*, for larvae and adult mosquitoes respectively.

DISCUSSION

Comparing our data with the previous studies about emathofagous arthropods of Circeo National Park [17], the mosquito species composition recorded during the present study is different. In fact, we found 5 species (*An. maculipennis*, *Ae. albopictus*, *Oc. communis*, *Cs. longiareolata*, and *Ur. unguiculata*) not reported in the above cited study, whereas 4 previously reported species (*An. claviger, Ae. berlandi, Ae. mariae, Orthopodomyia pulchripalpis*) were not found here. Differences in sampling technique may explain such discrepancies, for example our traps were placed mostly in poultry pens and this could have selected ornithophilic species. Moreover, as *Culex* spp. are known to be attracted to light traps [18], the amount of *Cx. pipiens* adults captured in site E and F could depend on this factor. In contrast, the small number of *Anopheles* spp. could be due to a low attraction toward the light of these mosquitoes [18] or to their different behaviour. *Anopheles* spp. larvae were probably not found because sampling

Table 1

Total number (No.) of mosquito specimens as larvae and adult per site and corresponding percentages per site (%); monthly occurrence of larvae (collected in 2004) and of adults collected (in 2003-04) are indicated in Roman fonts

	Larvae							Adults						
Species	Site A	Site B	Site C	Site D	No.	%	Months	Site A	Site B	Site E	Site F	No.	%	Months
Aedes albopictus			3		3	0.79	VII, IX		1	2	1	4	0.5	IX, X
Aedes communis				12	12	3.13	VI, X, XII					0	0	
Aedes vexans	14				14	3.68	V	3				3	0.4	V
Anopheles plumbeus					0	-		11				11	1.5	II
Anopheles maculipennis					0	-				2		2	0.2	X, XII
Ochlerotatus caspius					0	-		4			4	8	1.1	V, X
Ochlerotatus detritus		17	1	108	126	33.16	I, III, V, VI, X, XII	1		30	14	45	6.3	III, IV, VI, X
Ochlerotatus geniculatus					0	-		1				1	0.1	V
Ochlerotatus rusticus	6				6	1.58	I, III	8				8	1.1	IV, V, VI
Culex pipiens		56	28	27	111	29.21	IV-X, XII		2	523	98	623	87.3	I, IV-VII, IX, X, XII
Culiseta annulata	8	6	67	3	84	22.11	I, III-X, XII			4	4	8	1.1	VII, X, XII
Culiseta longiareolata			1		1	0.6	VII					0	0	
Culiseta litorea	1					0.26	III					0	0	
Culiseta subochrea	1		1			0.53	III, IV					0	0	
Uranotaenia unguiculata		6	14		20	5.26	VI, VII-X					0	0	

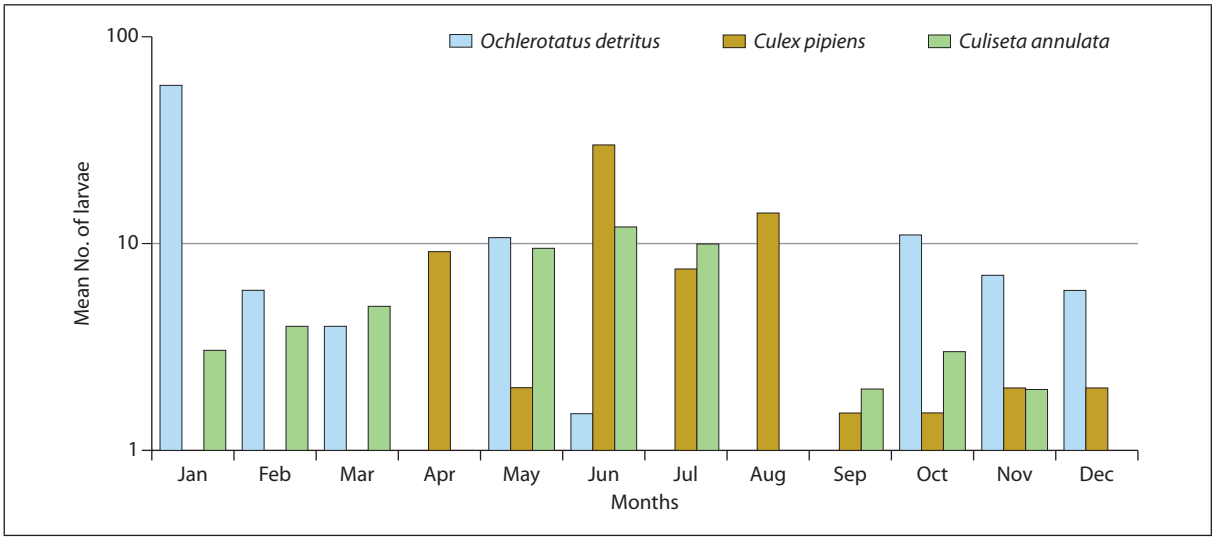

Figure 1

Seasonal trend of mosquito larvae expressed as monthly mean number of *Ochlerotatus detritus, Culex pipiens* and *Culiseta annulata*, caught in all the selected breeding sites, in 2004.

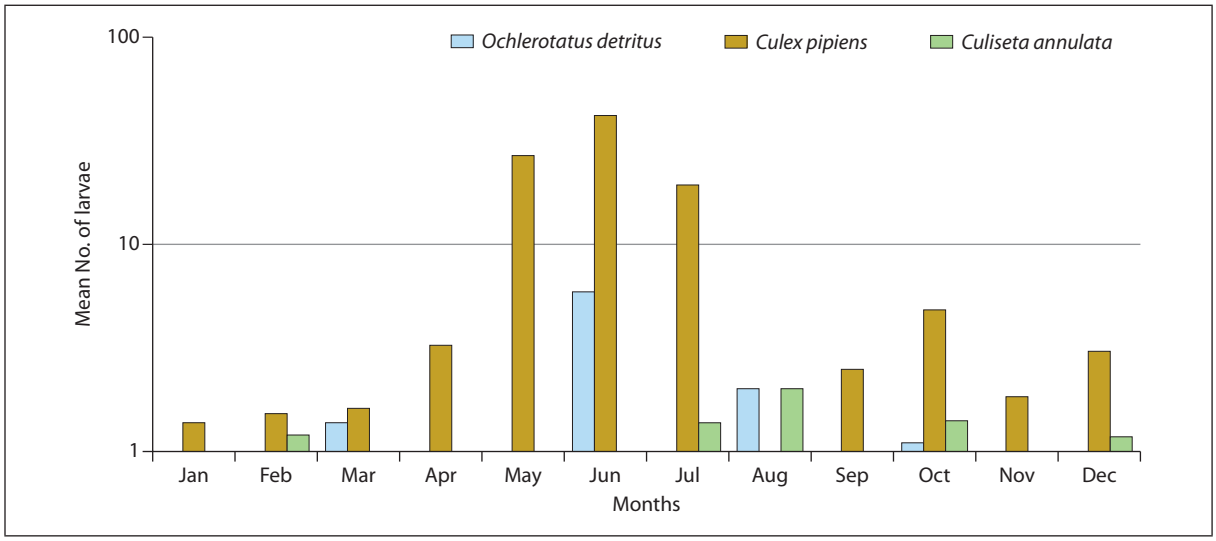

Figure 2
Seasonal trend of adult mosquitoes expressed as monthly mean number of *Ochlerotatus detritus, Culex pipiens* and *Culiseta annulata*, caught in all the selected breeding sites, in 2004.

was not carried out in their favourite habitats, such as tree holes for *An. plumbeus* or small natural and artificial pool for *An. claviger*, the two species reported as early stages by Rivosecchi and Stella in 1973 in "Selva del Circeo" [17]. The most represented species in our collections was *Cx. pipiens,* found in sites E, F for adults and sites B, C, D for larvae (*Table 1*). This species, known as the main West Nile virus vector, still remains one of the most abundant species in the monitoring activity carried out in the last years for the entomological surveillance toward this disease in Italy [19]. *Cx. pipiens* belongs to a complex occurring in Italy with two ecological forms or biotypes, *Cx. pipiens* f. *pipiens* and *Cx. p.*f.*molestus* Forskal, 1775, that differ only in behavioural and physiological characteristics. In such collection we considered *Cx. pipiens* specimens as belonging to the rural form in absence of data from molecular analyses and on the base of the context of the study area. The abundance of *Oc. detritus* respect to *Oc. caspius,* species sometimes occurring together [20], could be due to its preference for saline stagnant water bodies, common situations in the study area. Moreover, the large amount of larvae of *Oc. detritus* collected in January would confirm this species trait of overwintering at different development stages, with early adults emerging in spring and biting human persistently [21]. *Ae. albopictus*, the Asian tiger mosquito, was obviously absent in the past researches in this area, as accidentally introduced in Italy in 1990 [22] but now belonging to Italian culicid fauna,constitutes another species relevant from a public health point of view as vector of human viruses such as Chikungunyia, Dengue, Yellow fever, and others [23] and of canine filariosis [24]. In fact, formerly an annoying insect, since 2007 this mosquito showed its aspect as vector of Chikungunyia virus in Italy, during the outbreak in province of Ravenna [23, 25]. Regarding *Ae. vexans*, species considered widely distributed in Italy and in the Paleartic Region [28], its record should be considered noteworthy as very few data are available about its punctual distribution in Central Italy.

The site A showed the higher number of species (*Table 1*), probably for the preserved natural conditions offering various larval breeding sites until now, such as ponds and tree holes. In fact, among the seven species recorded in site A, *Ae. vexans*, *An. plumbeus*, *Oc. geniculatus*, *Oc. rusticus*, *Cs. litorea*, and *Cs. subochrea* often select such small water receptacles occurring in wooden environments. The findings of *Cs. litorea* and *Cs. subochrea*, even if subsequently collected in this area [26], constitute interesting faunistic data as here reported for the first time in Circeo National Park. *Cs. litorea* in particular is known as mainly occurring in Italy in central and southern regions and in the main island, as the vicariant species of *Culiseta morsitans* (Theobald, 1901) [20]. Both these species find the optimal larval habitat in brackish ponds often in association with *Oc. detritus*.

CONCLUSIONS

Circeo National Park seems to support a rich mosquito fauna in terms of number of species. Differences in species composition respect to previous studies could reflect environmental changes determined by human activities, mainly hydrological transformations, reclamations and massive employment of organochlorine insecticides. Our results highlight that the screening of mosquito fauna in protected natural areas can be a useful base to acquire data about the occurrence of potential vectors of pathogens, like in the case of West Nile Disease spreading in Europe in the last decades and in Italy too. According to this perspective, the surveillance of the Italian mosquitoes needs more efforts in order to implement the knowledge of their bionomics also within a monitoring activity of mosquito-borne diseases in general.

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Conflict of interest statement

No conflict of interest.

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